

9-5-1990

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Hojo, Teruyuki (1990) "Scanning Electron Microscopic Quantitative Study of Changes with Age in Closing Pattern of Openings of Dentinal Tubules on Worn Occlusal Surfaces of Japanese Permanent Mandibular Incisors," *Scanning Microscopy*. Vol. 4 : No. 4 , Article 16.

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**SCANNING ELECTRON MICROSCOPIC QUANTITATIVE STUDY OF CHANGES WITH
AGE IN CLOSING PATTERN OF OPENINGS OF DENTINAL TUBULES ON WORN
OCCLUSAL SURFACES OF JAPANESE PERMANENT MANDIBULAR INCISORS**

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(Received for publication May 3, 1990, and in revised form September 5, 1990)

Abstract

Age-related changes in the closing of dentinal tubules of Japanese permanent mandibular incisors were surveyed using a non-destructive replication technique suitable for SEM analysis at the magnifications of up to X3,000. The areas of openings of dentinal tubules on occlusal surfaces decreased significantly between a 20-39-year age group and a 40+ age group, but the number of the openings tended to decrease. Heavy toothwear on occlusal surfaces of these Japanese permanent incisors was evident even at 27 years of age. A subject of 30 years of age showed surprisingly small number of the opening of dentinal tubules on occlusal surface. The occlusal surfaces became smoother in 40-59-year age group as closure of dentinal tubule of openings increased. Early heavy toothwear and early closure of the openings of dentinal tubules may be related to Japanese ethnic diet such as dried hard sardines whole which contain hard bones. Japanese often crush shells of crabs and shrimps with their incisors and canines as they eat.

Key Words: SEM quantitative study, age change, closing pattern of opening of dentinal tubule, occlusal surface, Japanese permanent incisor.

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Introduction

SEM analyses have shown that microwear on the occlusal surfaces of teeth may be related to ethnic differences in diet and dietary behavior (Gordon, 1986; Hojo, 1989; Peters, 1982; Rensberger and Krentz, 1988; Rose, 1986; Teaford, 1988; Walker, 1979).

Incisors of Japanese often show heavy wear and rough occlusal surfaces before 40 years of age. The toothwear becomes slightly heavier with the advance of age and occlusal surfaces become smooth. Many Japanese like small dried hard fish such as whole sardines. This is, especially true for people living in coast districts who take these hard fish almost every day. These small dried hard fish contain bones including vertebrae, ribs, and skulls. Japanese crunch the bones whole by using their incisors. This practice evidently contributes to heavy incisal wear, as dentin is readily observed on Japanese incisors.

Dentinal tubules of Japanese teeth have been studied by SEM (Kimura et al., 1988; Sukekawa et al., 1987; Yamazaki, 1981), but none of these studies have looked at age changes in dentinal tubules.

Mendis and Darling (1979a, b) noted that dentinal tubules on worn occlusal surfaces were all completely occluded by a smooth homogeneous material similar to peritubular dentin.

As attrition and microwear of teeth may be related to ethnic differences in diet, it was felt that SEM analysis study of age changes in dentinal tubules on Japanese worn teeth might be related to ethnic differences in diet.

Materials and Methods

As shown in Table 1, ten first mandibular incisors of Japanese from coastal districts of Kyushu were examined. For this study subjects were

three age groups; 20-39-years of age (N=5), 40-59-years of age (N=2) and over 60-years of age (N=3). In each case a two-stage replication technique was used to make a high resolution cast of the teeth. First, an impression was taken using a polysiloxane impression material (Coltene, "Light-Body") (Beynon, 1987). Second, a low-viscosity epoxy resin (Ciba-Geigy, "Araldite") was used to make the positive cast (Hojo, 1989).

These casts were sputter coated with a 10nm film of platinum and observed with ABT SX-40A scanning electron microscope (Akashi-Beam Technology, Tokyo Japan) at magnifications ranging from X50 to X3,000 and at 20 or 25kV. Areas of the openings of dentinal tubules on occlusal surfaces were measured at X3,000 with a digitizer (IMS-UNISCIENCE made in Fukuoka city Japan). For each incisor two measurements were recorded; (1) the number of openings per $820\mu\text{m}^2$, and (2) the mean area of each opening. These measurements were then compared between age groups.

Results

All specimens showed large or small dentinal surfaces. The number of openings per $820\mu\text{m}^2$ and the mean and its standard deviation are shown in Table 1.

The number of the openings of dentinal tubules tended to decrease with the advance of age (Table 2), and closed openings of dentinal tubules tended to increase from the youngest age group to the oldest.

As shown in Figures 1 - 6, subjects in 20-39-year age group showed large areas of dentin and many openings of dentinal tubules on their first incisors.

In the group aged 40-59 years, dentinal surfaces were smoother (Figures 7-8) than those of the group aged 20-39 years.

In the group aged over 60 years, dentinal surfaces became still smoother and many closed dentinal openings were observed (Figures 9-11). The closing patterns of openings were similar to those noted by Mendis and Darling (1979a, b, Yamazaki (1981), and Kimura et al. (1988). The means of areas of the opening of dentinal tubules were the smallest among the materials in this study. An analysis of variance showed there was a significant difference between the 3 age-samples (Table 3). However, due to small sample sizes, subsequent multiple comparison tests could not determine the location, within the samples, of the significant differences. However, if the data sample is restructured into 2 age-groups (20-39 & > 40 years), t-tests indicate a significant difference in mean areas between 2 age-groups ($t = 3.403$, $p < 0.01$, Table 4).

Table 1. The number, the mean and the standard deviation of area of the openings of dentinal tubules per $820\mu\text{m}^2$ in Japanese.

Age group	No.	Age	N	Mean	SD
Y1 20-39	1	27	34	2.46 μm^2	1.49 μm^2
	2	29	11	3.01	1.78
	3	29	31	1.26	2.13
	4	30	37	1.26	0.94
	5	30	27	1.40	0.95
Y2 40-59	6	47	20	0.56	0.41
	7	48	33	1.22	0.66
Y3 over 60	8	60	24	0.20	0.25
	9	63	21	0.12	0.08
	10	72	13	0.32	0.27

Table 2. The number of the subjects, and the mean and the standard deviation of the number of the dentinal tubule openings per $820\mu\text{m}^2$ in each age group.

Age group	N	Mean	SD
Y1 20-39	5	28.0	10.20
Y2 40-59	2	26.5	9.19
Y3 over 60	3	19.3	5.68

Y1-Y3: $t_0 = 1.531 < t = 2.447$ ($p < 0.05$, $f = 6$), insignificant

Discussion

While many studies have dealt with patterns of microwear on enamel occlusal surfaces, the natural patterns and age changes of dentinal occlusal surfaces have not been studied.

This study surveyed and measured areas of the openings of dentinal tubules on occlusal surfaces of Japanese permanent first incisors by using an accurate two-stage replication technique and higher magnification (X3,000) scanning microscopy.

These Japanese permanent first mandibular incisors showed heavily worn occlusal surfaces even in young subjects aged from 27 to 30 years (Figures 1 - 6). Openings of dentinal tubules on the occlusal surfaces began to close in the same subjects aged 27-30 years. In the middle aged group, 40-59 years of age, the dentinal occlusal surfaces became

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Table 3. An analysis of variance of the areas of the openings of dentinal tubules among three age-groups.

	Sum of Square	f	Mean Square
Between groups	17.43	2	6.67
Within groups	6.67	7	0.953
Total	24.01	9	

$F_0 = 9.0976 > F = 4.737$ ($p < 0.05$, $f_1=2$, $f_2=7$) significant

Table 4. T-tests of the restructured data of the dentinal tubule openings between two age-groups.

Age group	N	Number Mean	SD	Area Mean	SD
20-39	5	28.0	10.20	1.88um ²	0.81um ²
> 40	5	22.2	7.26	0.48	0.44

Number: $t_0=1.036$, insignificant

Area: $t_0=3.403 > t=3.355$ ($p < 0.01$, $f=8$), significant

smoother and the number of closed dentinal tubule openings increased (Figures 7-8). In over 60-year age group, the number of the openings of dentinal tubules tended to decrease as dentinal surfaces became smoother (Figures 9 - 11). Furthermore, areas of openings of dentinal tubules became smaller.

Admittedly, these results are based on analyses of small samples, and there is certainly individual variation in some measurements (e.g., the small number of openings for individual #2 in the 20-39 year age group). However, other differences are still significant, despite the sample limitations.

Mendis and Darling (1979b) showed the many partly open dentinal tubules from a subject aged 76 with slight attrition. Yamazaki (1981) showed many closed dentinal tubule openings on worn occlusal surface of Japanese teeth. But the present study showed a significant decrease of the areas of openings of dentinal tubules on the heavily worn occlusal surfaces of Japanese permanent incisors with the advance of age. The combination of early heavy toothwear and smooth dentinal occlusal surfaces in advanced age-group may be related to crunching hard dried fish of Japanese in coast districts.

Further support for this interpretation may be found in the fact that peritubular-dentinlike matrices and intratubular deposits were observed around partly or closed openings of dentinal tubules from the 20-39 age group in this study. As Kimura et al. (1988) and Yamazaki (1981) noted, dentinal tubule openings on worn occlusal surfaces

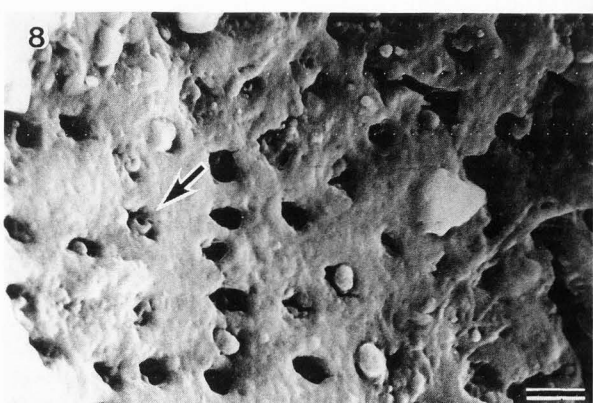
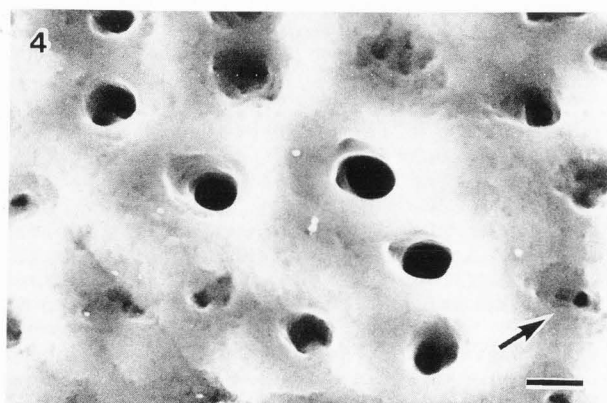
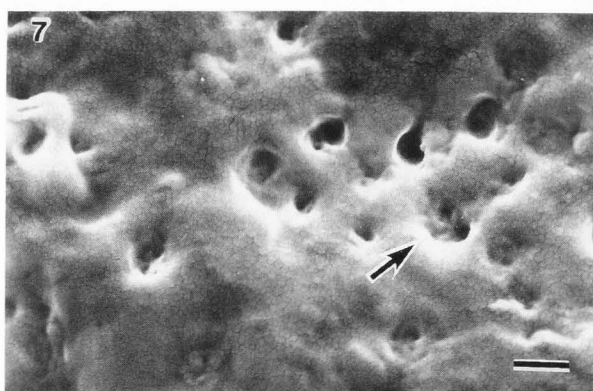
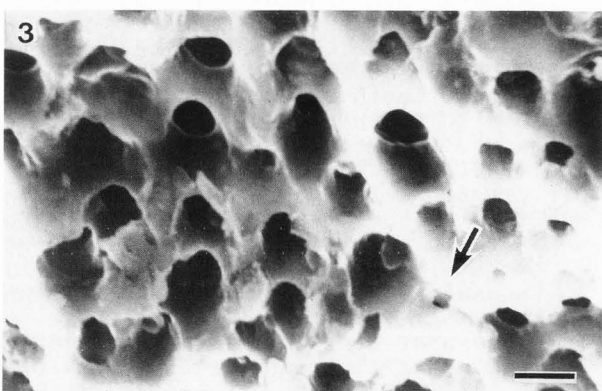
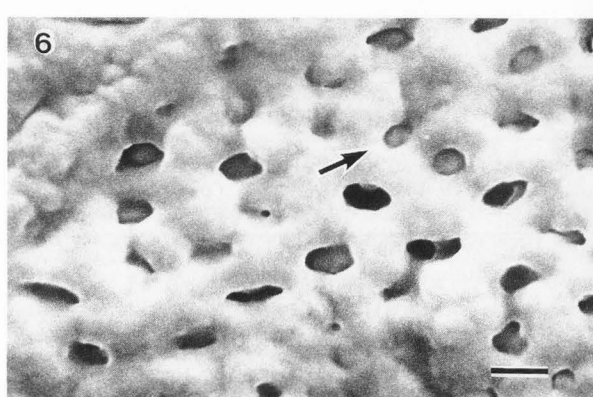
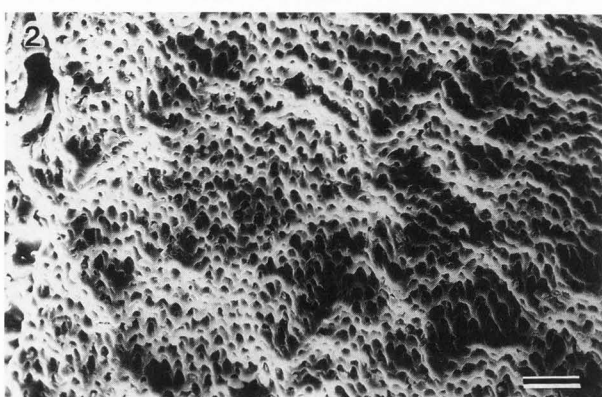
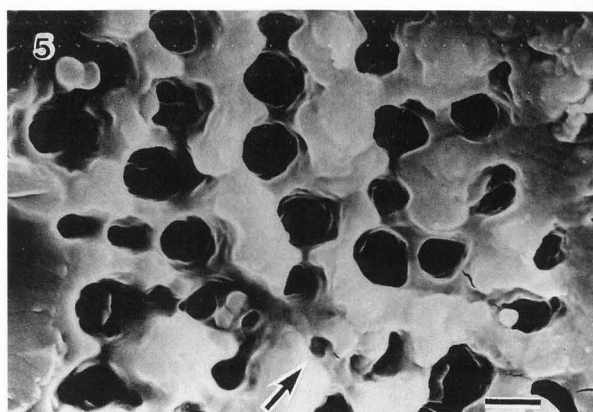
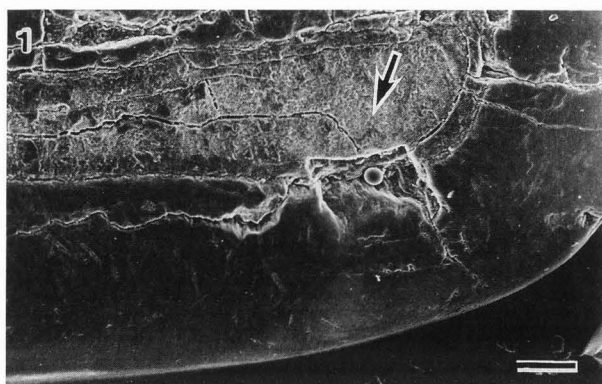
may become calcified through exchange of ions in saliva of the mouth. Thus, early closing of dentinal tubule openings observed on heavily worn teeth in this study may be related to these both a hard diets and the consumption of food items full of calcium.

Acknowledgments

I wish to thank both Dr. John M. Rensberger, Dept. Geol. Sciences and Burke Museum DB-10, Univ. Washington, and Dr. Mark F. Teaford, Dept. Cell Biology and Anat., The Johns Hopkins Univ., Sch. Med. for their suggestions, comments, and checking English sentences, and also for Dr. Mark F. Teaford for his suggestion in making casts of teeth.

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Figure 1. Worn occlusal surface of mesiolabial half of the first incisor of No. 1 aged 27. Dentinal surface was shown. Scale bar = 250 μ m.

Figure 2. Higher magnification of the arrow-pointed area in Figure 1. Occlusal surface is rough. Scale bar = 25 μ m.

Figure 3. Higher magnification of the central area in Figure 2. The arrow points a narrowed dentinal tubule opening surrounded with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.

Figure 4. Higher magnification of dentinal tubule openings on occlusal surface of No. 2 aged 29. More than ten almost closed dentinal openings were observed. An arrow shows one of them surrounded with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.

Figure 5. Dentinal tubule openings on occlusal surface of No. 3 aged 29. Narrowed dentinal tubule openings (arrow) surrounded with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.

Figure 6. Dentinal tubule openings on occlusal surface of No. 4 aged 30. Rough dentinal surface with round projections. Closed dentinal tubule openings with partly closed openings. The openings appear to be smaller. An arrow points to one of almost closed openings with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.

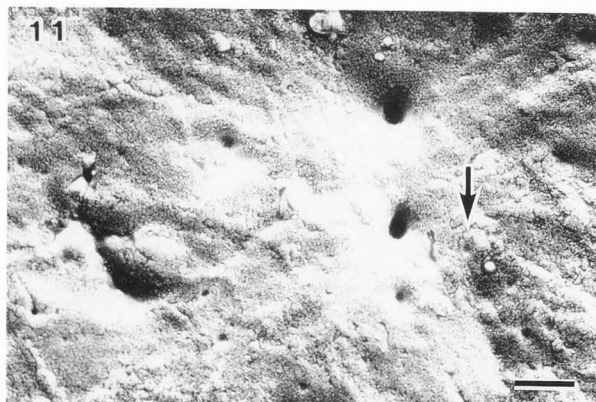
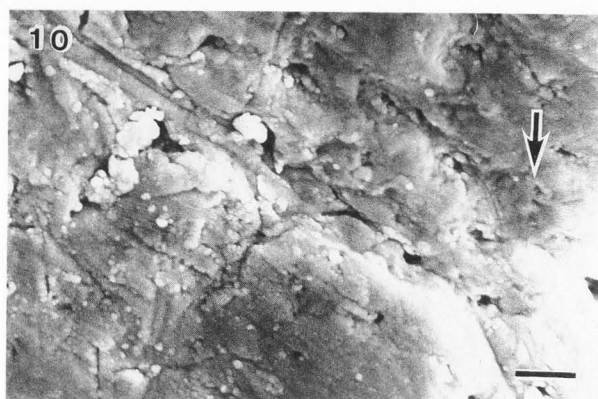
Figure 7. Dentinal surface of No. 6 aged 47. Narrowed dentinal tubule openings are irregularly shaped on rough dentinal surface. An arrow shows peritubular-dentinlike matrices and intratubular deposits at one of partly closed dentinal tubule openings. Scale bar = 4 μ m.

Figure 8. Smooth dentinal surface of No. 7 aged 48. An arrow shows peritubular-dentinlike matrices and intratubular deposits at a ring-shaped dentinal tubule openings. Scale bar = 4 μ m.

Figure 9. Smooth dentinal surface of No. 8 aged 60. Note small dentinal tubule openings and a number of low circular cones. The arrow points an almost closed dentinal tubule opening circled with peritubular-dentinlike and intratubular deposits. Scale bar = 4 μ m.

Figure 10. Dentinal surface of No. 9 aged 63. Note narrowed dentinal tubule openings. The arrow points a closed dentinal tubule opening surrounded with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.

Figure 11. Smooth dentinal surface of No. 10 aged 72. Note a few narrowed dentinal tubule openings. The arrow points a closed dentinal tubule openings with peritubular-dentinlike matrices and intratubular deposits. Scale bar = 4 μ m.



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